

Original article

Airway inflammation and cough sensitivity in cough-variant asthma

Background: Mechanisms underlying cough and bronchoconstriction in patients with cough-variant asthma (CVA) are not well established. Differences in location or degree of activation of eosinophils and allergic cytokines have been suggested as the likely causes. To address this issue, we have carried out a comparative study of airway inflammatory markers between patients with CVA and classic asthma (CA). The relationship between these markers with airway hyperresponsiveness (AHR) and cough sensitivity has also been studied.

Methods: Twenty-seven non-smokers and steroid-naïve patients with CVA (12) and CA (15) were examined. Capsaicin challenge, histamine bronchoprovocation test, nitric oxide levels in exhaled air and sputum induction were performed in all of them. Differential cell sputum recount and supernatant concentrations of eosinophil granule-derived cationic proteins (ECP), interleukin (IL)5, IL8 and tumour necrosis factor (TNF)- α were also measured.

Results: There were no significant differences in either the inflammatory pattern of soluble markers or differential cell counts between CA and CVA. Histamine PC20 was correlated with IL-5 in CVA, whereas it was associated with sputum eosinophilia in CA. Cough sensitivity (log C5) and histamine PC20 were inversely related in CA.

Conclusions: Although the pattern of inflammatory sputum markers in patients with asthma and cough-variant asthma is similar, its relation with bronchial hyperreactivity and cough sensitivity is different in each group.

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Key words: airway hyperreactivity; airway
inflammation; asthma; cough-variant asthma.

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Accepted for publication 10 April 2004

In 1979, Corrao et al. (1) described a group of patients with hyperreactive airways and excellent response to bronchodilators whose only presenting symptom was cough. This clinical variant of asthma was called cough-variant asthma (CVA). Since then, diverse reports (2, 3) have shown that a high percentage of those patients, nearly 30%, develop symptoms of wheezing and dyspnoea in the following 3–5 years, completing the criteria necessary to diagnose typical or classic asthma (CA).

The mechanisms underlying the symptomatic prevalence of cough in CVA are not well established. Differences in airway hyperresponsiveness (AHR), sensitivity of cough receptors and wheezing threshold between both variants of asthma, have been referred in the literature as possible causes (4, 5). Cough response to inhaled capsaicin, which stimulates C-fibre endings in airways, is increased in patients with asthma (6), but it is also raised in subjects with asthma-like symptoms induced by inhalation of chemical irritants (7, 8). Lack of association between cough sensitivity and bronchoconstrictor responsiveness, suggests that the mechanisms underlying cough production in CVA could not be related to those modulating airway muscular tone (9).

Some experimental studies have shown that human eosinophil granule-derived cationic proteins (ECP) can stimulate vagal afferent C-fibres in the lungs and elicit both cough and bronchoconstrictor reflexes (10). Tachykinins localized in the nerve endings of the afferent C-fibres and liberated after eosinophilic activation can modulate the inflammatory process, contributing to the different pathophysiology and symptoms of both entities (11). As in CA, marked increase in sputum and tissue eosinophilia levels have been observed in the airways of patients with CVA (12) and eosinophilic bronchitis (13). To our knowledge, the different modulating effects of allergic cytokines in the diverse expressions of cough and bronchoconstrictor reflexes in both groups have not been clearly investigated. Of these allergic mediators, IL-5 is the predominant cytokine associated with eosinophilic inflammation and is of key importance in the pathogenesis of asthma. Likewise, isolated epithelial cells from asthmatics have shown an increased production of interleukin (IL)-8 and tumour necrosis factor (TNF)- α .

Therefore, we have carried out a comparative study of some airway inflammatory markers (sputum cell counts, exhaled nitric oxide and sputum ECP, IL-5, IL-8 and

TNF- α) between patients with CVA and CA. The relationship of these markers with AHR and cough sensitivity has also been examined.

Material and methods

Study subjects

Twenty-seven patients selected from our outpatient clinic participated in the study. Fifteen of them were regarded as having CA and 12 CVA. Patients with CVA had been referred to our clinic for study and treatment of unexplained cough lasting >8 weeks. An exhaustive diagnostic evaluation that included medical history (cough characteristics, respiratory and digestive symptoms), physical examination, chest radiography, spirometry, bronchodilator test, histamine challenge, 24-h oesophageal pH measurement and otorhinolaryngologic evaluation was undertaken. The International Union against Tuberculosis and Lung Diseases (IUATLD) questionnaire was used to assess symptoms of shortness of breath, cough, chest tightness and wheezing. Reproducibility of the test has been previously tested (Table 1).

Diagnosis of CVA was established according to the following criteria (3):

- absence of a history of wheeze or dyspnoea;
- normal physical examination;
- positive histamine challenge (PC₂₀ histamine < 8 mg/dl);
- relief of cough with bronchodilator therapy;
- absence of other causes of cough.

Patients with CA were recruited from the same outpatient clinic. They were referred with a history of at least 12 months of episodic wheezing, cough, and shortness of breath. All of them were non-smokers and none were taking inhaled steroids. Diagnosis was established according to the National Asthma Education Programme (NAEPP) criteria (14). Histamine challenge test was performed following Cockcroft's method (15).

Table 1. Baseline characteristics of 27 patients with asthma

Data	CA	CVA	P-value
n	15	12	
Age (years)	29 (11)	31 (11)	NS
Gender (male/female)	5/10	3/9	NS
Duration (months)	34 (14)	24 (23)	NS
Cough (%)	25	100	<0.05
Chest tightness (%)	15	14	NS
Wheezing (%)	80	0	NS
Shortness of breath (%)	70	25	NS
Atopy (%)	66	25	<0.05
Rhinitis (%)	40	50	NS
FEV1 (l/min)	3.3 (0.8)	2.90 (0.4)	NS
FEV1 (%pred)	95 (5)	92 (9)	NS
FEV1-post-capsaicin	3.38 (0.8)	2.89 (0.43)	NS
FEV1-postbd (%)	15 (2)	17 (3)	NS
Histamine PC ₂₀ (mg/ml)	3.2 (2.6)	2.8 (1.9)	NS
NO _{ex} (ppb)	10 (9)	5 (2)	NS
C2 (μ M)	116 (169)	64 (77)	NS
C5 (μ M)	230 (188)	193 (164)	NS

CA: classic asthma; CVA: cough-variant asthma; C2: concentration of capsaicin that causes two coughs; C5: concentration of capsaicin that causes five coughs.

NS: not significant. * $P < 0.05$

Data expressed as mean (SD) or percentage of patients.

Study design

The design was a cross-sectional observational study comparing patients with CA and CVA. All patients attended the laboratory on two consecutive days. On the first day, we performed the skin-prick tests, spirometry, and determined the concentration of nitric oxide in exhaled air (NO_{ex}). Exhaled NO was measured using a chemiluminescence analyser (Logan Research 2000, Rochester, UK) according to the method described by Kharitonov et al. (16). After that, patients were challenged with capsaicin. Twenty-four hours later, a sputum induction with hypertonic saline solution was performed.

Allergen skin sensitivity was measured by skin-prick test to a battery of 11 common airborne allergen extracts including house dust mites, moulds, tree pollens, parietaria, animal danders, a negative (saline solution) and a positive (histamine) control. Atopy was defined by the presence of one or more positive tests (wheal diameter > 3 mm).

The institutional review board approved the study, and written consent was obtained from all patients once the purpose of the study had been discussed with them.

Capsaicin challenge. Cough receptor sensitivity to capsaicin was examined using a modified version of the method described by Fujimura (17). The challenge was performed by single-breath inhalation of doses increasing from 0.49 to 500 μ M. Subjects were asked to inhale deeply once at 2-min intervals. The number of coughs during the first 30 s after each dose was counted. The challenge was terminated when the subject coughed five or more times (C5) or the maximal dose of capsaicin was attained. In subjects who did not cough, a value of C5 of 500 μ M was assumed. The concentration of capsaicin inducing two coughs (C2) was also recorded. Subjects were unaware that coughing was the specific point of research.

Sputum induction. Sputum was induced by inhaling hypertonic saline solutions (3, 4 and 5%) generated by an ultrasonic nebulizer (NE-U03; OMRON Co., Tokyo, Japan). For the purpose of this study, the sample was divided in two aliquots. One aliquot was processed as previously described by Pizzichini (18). The other aliquot was used for the analysis of fluid-phase mediators. Given that dithiothreitol (DTT) could modify the biological activity of cytokines, this sample was only treated with phosphate-buffered saline (PBS). After centrifugation, the cell pellet was discharged and the supernatant was stored at -70°C until analysis.

Quantitative determinations of interleukins were obtained using commercially available kits. IL-8 and TNF- α were measured using a solid-phase sandwich enzyme-amplified sensitivity immunoassay (EASIA) (Biosource Europe S.A., Nivelles, Belgium). Absorbance of the samples in the microtiter plates was read at 450 nm wavelength (Labsystems Multiskan EXIL-8; Labsystems Oy, Helsinki, Finland). Concentrations were determined by lineal interpolation of the standard curve. The minimal detectable concentration of IL-8 and TNF- α were 0.7 and 3 pg/ml, and coefficients of variation were 3.5 and 3.7, respectively. Interleukin-5 was measured using a solid-phase sandwich enzyme linked-immunosorbent assay kit (ELISA) (Biosource International) with human IL-5 monoclonal antibodies. The sensitivity of the procedure as suggested by the manufacturer is 4 pg/ml (minimum detectable dose) and the reproducibility (intraassay coefficient of variation) is 2.5.

The concentration of ECP in the supernatant was determined using fluorimmunoassay (UniCAP system; Pharmacia AB, Uppsala, Sweden). The limit of detection was 2.0 pg/l.

Statistical analysis. The sample-size calculation ($\beta > 80\%$ and $\alpha 0.05$) was based on the results of previous studies which estimated significant differences in sputum markers between asthmatics and

healthy individuals (19). Data values were expressed as mean and standard deviation. Differences in clinical or functional outcomes and sputum markers between patients with CA and CVA were assessed using a non-parametric one-way analysis of variance (Kruskal–Wallis test) or the chi-squared test, when indicated. Relationships between C5 and histamine PC₂₀ and cytokines, ECP, or differential cell counts were evaluated by calculating the Spearman coefficient of correlation. *P*-values of <0.05 were considered statistically significant.

Results

Twenty-seven patients (eight men and 19 women) participated in the study. Clinical and functional characteristics of patients in the study are shown in Table 1. No significant differences between patients with CA and CVA, in terms of pulmonary baseline function values, concentration of NO_{ex}, histamine PC₂₀ and capsaicin thresholds (C₂ and C₅) were found. However, the frequency of atopy was significantly higher in patients with CA (66% vs 25%). Although there was a small trend in increased values of eosinophils [9 (5)% vs 2 (2)%] and ECP [91 (40) mg/ml vs 64 (6) mg/ml] in sputum of patients with CA compared with CVA, these differences were not statistically significant (Table 2). Total cell counts and differential percentage of lymphocytes, neutrophils and macrophages in sputum were similar in both groups (Table 2). Concentrations of IL-8, IL-5 and TNF- α in the supernatant did not differ significantly from each type of patients (Fig. 1).

A significantly inverse correlation between AHR to histamine and cough sensitivity to capsaicin was observed in patients with CA ($r = -0.54$; $P = 0.03$). In this group, histamine PC₂₀ values were also inversely correlated with the percentage of sputum eosinophils ($r = -0.72$; $P = 0.01$). In patients with CVA, a significant correlation was found between histamine PC₂₀ values and IL-5 supernatant concentrations ($r = 0.69$; $P = 0.01$) (Fig. 2A). We did not find any significant relationship between ECP, IL-8 or TNF- α and histamine PC₂₀ in either group (Fig. 2B,C).

Table 2. Results of induced sputum in 27 patients with asthma

	CA	CVA	<i>P</i> -value
Cells			
Total counts ($\times 10^6/\text{mm}^3$)	2.3 (0.5)	1.8 (0.4)	NS
Macrophages (%)	47 (23)	49 (36)	NS
Lymphocytes (%)	6 (8)	5 (4)	NS
Neutrophils (%)	41 (30)	43 (37)	NS
Eosinophils (%)	9 (5)	2 (2)	NS
Supernatant			
ECP (mg/ml)	91 (40)	64 (6)	NS
IL5 (ng/ml)	206 (72)	270 (226)	NS
IL8 (ng/ml)	643 (265)	680 (270)	NS
TNF- α (ng/ml)	88 (112)	135 (185)	NS

CA: classic asthma; CVA: cough-variant asthma; NS: not significant.

* $P < 0.05$. Data expressed as mean (SD).

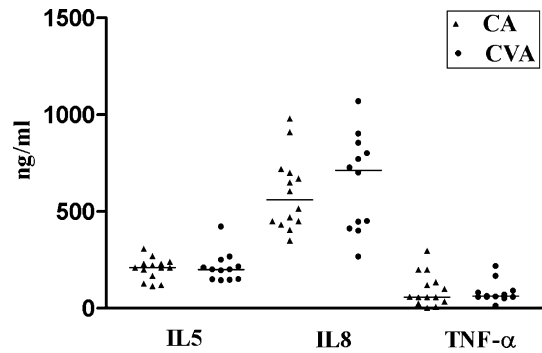


Figure 1. Values of IL-8, IL-5 and TNF- α in induced sputum of patients with cough-variant asthma (●) and classic asthma (▲).

As the proportion of patients with atopy was higher in CA than in CVA, our results were analyzed after adjusting by atopic and non-atopic status. Differential cell counts in sputum, ECP and cytokines values were similar in atopic and non-atopic patients. Like in CA, histamine PC₂₀ values were correlated with the percentage of sputum eosinophils in atopic subjects ($r = -0.72$, $P = 0.04$).

Discussion

This study shows that patients with CVA and CA share a similar airway inflammatory marker's profile. Despite this, the contribution of eosinophilic inflammation and sputum cytokines to cough sensitivity and AHR shows some differences between both clinical variants.

The relationship between cough receptor sensitivity and bronchial reactivity (BHR) cough sensitivity in CA and CVA has not been previously well investigated. Previous studies have shown that sensitivity of capsaicin cough receptors is increased in stable asthmatics compared with normal subjects (20). However, this cough hypersensitivity has also been associated with several causes of chronic cough as: angiotensin-converting enzyme inhibitors, chronic obstructive pulmonary diseases (COPD), viral infections, or gastroesophageal reflux (21, 22). In the present study, cough sensitivity to capsaicin is similar in patients with CVA and CA. However, the relationship between cough receptor sensitivity and BHR was significantly different between them. In patients with CA, BHR was inversely related with cough sensitivity. There is not a clear explanation for this finding. Bronchoconstriction is mediated by rapidly stretch receptors and c-fibres, whereas cough-reflex is only stimulated by rapidly stretch receptors. It has been postulated that eosinophilic inflammation could modify these separate afferent pathways. Other possibility is that patients with CVA were poor perceivers of narrowing induced by histamine. In this case, cough induced by bronchoconstriction would not be

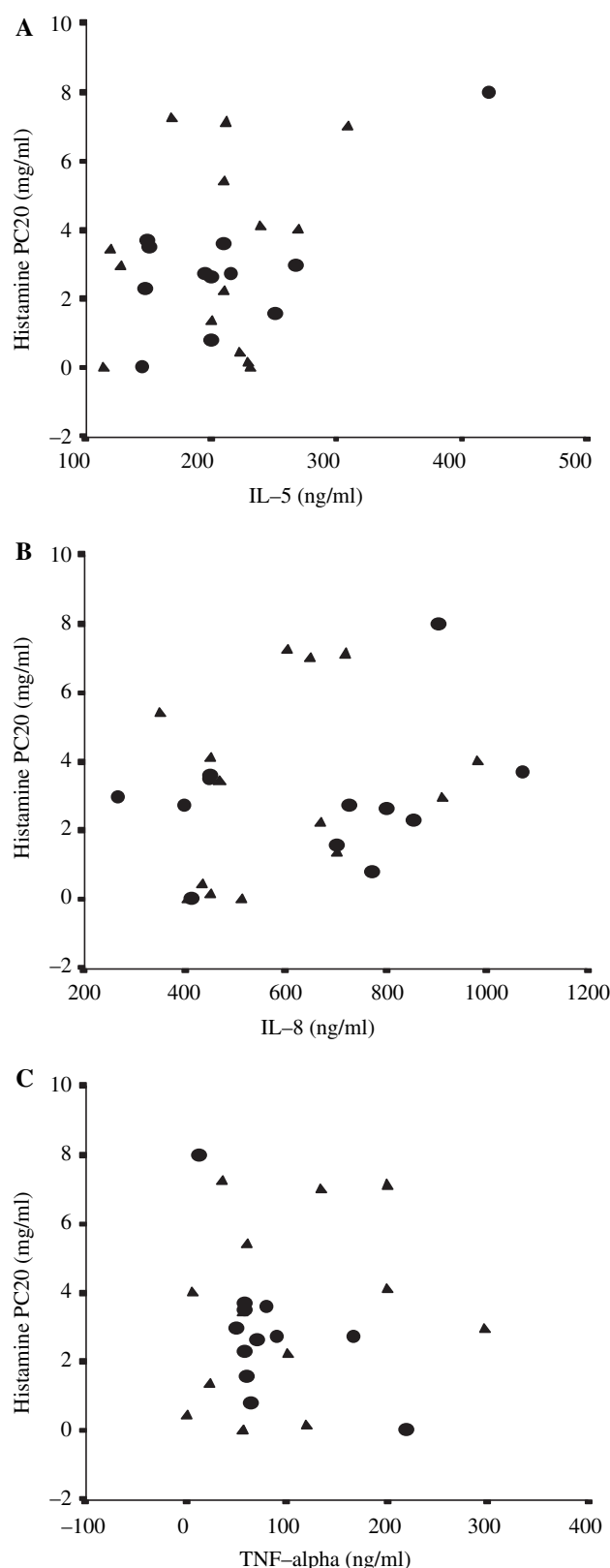


Figure 2. Relationship between PC20 histamine and values of IL-5 (A), IL-8 (B), and TNF- α (C) in induced sputum of patients with cough-variant asthma (●) and classic asthma (▲).

well perceived and it would explain the lack of association between BHR and cough sensitivity in this group. In our study, we did not assess cough provoked by histamine challenge and we can not reject this hypothesis.

Increased eosinophil counts in sputum have been reported by Niimi et al. (12) in patients with CVA. In our study, although the percentage of sputum eosinophils is increased in patients with CA with respect to CVA, the difference was not significant. Absence of statistically significant differences could be attributed to the small size sample and a type-2 error cannot be ruled out.

In patients with CVA, sputum eosinophilia was not correlated with cough sensitivity or BHR. By contrary, there was a closely correlation between severity of eosinophilic inflammation and BHR in CA. The precise mechanism by which eosinophils can induce BHR is not well known. Eosinophil granule proteins, which include ECP, are potent cytotoxic molecules capable of damaging airway epithelium. In our study, concentration levels of ECP in supernatant were similar in both groups. Other mechanisms involving different inflammatory mediators could be contributing. Sputum levels of prostanoïdes, bradykinin, histamine or cytokines are increased in patients with eosinophilic bronchitis (13) and chronic bronchitis (23). To our knowledge, this is the first study investigating the differences amongst some cytokines between patients with CA and CVA.

Interleukin-5, a protein produced by several different cells including CD4⁺ T cells, mast cells and eosinophils, is involved in eosinophilia development and activation (24). Increased expression of IL-5 mRNA in bronchoalveolar cells have been demonstrated in patients with chronic cough and asthma (25). In this study, association of BHR and IL-5 was observed in CVA. Cytokines IL-8 and TNF- α have also been studied. There is experimental evidence of both mediators being produced by airway epithelial cells following inflammatory stimuli, e.g. allergen exposure. Activation of capsaicin-sensitive nerves in human bronchial epithelial cell line was followed by increased transcription and release of these cytokines (26). Elevated levels of sputum TNF- α have been reported in asthmatic patients. In this study, we have not found differences in sputum concentrations of either IL-8 or TNF- α between patients with CVA and those with CA.

To summarize, our research supports the hypothesis that airway hyperreactivity and cough sensitivity in asthma are not always related and its different expression could be modulated by different inflammatory mechanisms.

Acknowledgments

This study was supported in part by grant SEPAR 2000 and RTIC G03/11 of FIS-Instituto Salud Carlos III.

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